

ESTIMATION OF DISTINCTION IN SEED FORMATION AND PRODUCTIVITY IN DIFFERENT GENOTYPES OF TOMATILLO (*PHYSALIS IXOCARPA* BROT.)

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Abstract

The main aim of the present study was to establish the responses in seed formation and seed production in several genotypes of tomatillo (*Physalis ixocarpa* Brot.) with a target to predict the seed yield and its realization. The experiments were carried out in Agricultural University of Plovdiv, Bulgaria with five genotypes with a different origin. The number of seed per fruit, the percentage of fully developed seeds, the linear seed sizes, the weight of 1000 seeds, germination energy, germination, main germination time, uniformity of germination, length of embryo root and hypocotyls, fresh weight and deviation in seedlings were investigated. The yields of fruit and seed also have been determinate. Significant variation in the numbers of seed per fruit as well as in the in weight of seeds per fruit was observed. High germination was an account of each genotype. The differences in seed production and in some sowing qualities between genotypes were registered. These results can be applied for the prediction of seed productivity and also in the determination of price during the realization of seed according to the genotype behaviors of the seed yield.

Key words: germination, seed formation, seed production, sowing quality, tomatillo.

INTRODUCTION

The genus *Physalis* is characterized by big diversity of annual and perennial species, according to various authors between 70 according to Christov (2010) and 110 (Skvorcova, 1997). Da Silva et al. (2016) also reported for the existence of a wide variety of species in *Physalis* and they claimed that in this genus comprising more than over one hundred species. The four main species as *Physalis peruviana* L., *Physalis pruinosa* L., *Physalis ixocarpa* Brot. and *Physalis pubescens* L. are the most popular (Moriconi et al., 1990; Crawford, 2004) and De Souza et al. (2017) emphasized that they cultivated more in the production of fruit used for several purposes. The tomatillo (*Physalis ixocarpa* Brot.) is most popular in Latin America and it is one of the new vegetable crops for Europe and for Bulgaria also. The information about the development and productivity of tomatillo is very limited (Díaz et al., 2005). Freyre and Loy (2000), investigating five tomatillo genotypes in New Hampshire, USA region, established statistical significance influence of

environmental conditions, related to fruit number and average fruit weight per genotype. In view of a higher seeds yield Peña-Lomelí et al. (2018) pointed out that the pollination is best to be accomplished on the second or third day of flowering.

Because the tomatillo reproduces primarily by seed, Barroso et al. (2017) pointed out that the investigations about seed maturation are very important to determine the most appropriate term of harvest, when the seeds are with a high physiological potential, even more so that the fruit until the end of the vegetation is completely enveloped by the sepals. According to them, the best time to harvest the seeds is 45 days after flowering. Similar studies have been carried out by Pérez et al. (2012), reporting that the influence of environmental conditions on germination and the physiological state of tomatillo seeds are poorly studied and with the highest quality are the tomatillo seeds, that have been harvested 55-65 days after flowering. In a previous publication, these authors (Pérez et al., 2008) also underline that the processes of seed development in the tomatillo have not been sufficiently studied, but here concluded that the highest viability has the seed at 42 days after flowering. De Souza et al. (2016), in

their studies, highlighted that the seeds of species of the genus *Physalis* are characterized by high storability. In view to establishing of the sowing qualities of tomatillo seeds, Martínez et al. (2006) indicates that it is appropriate to use a standard paper germination test and they have studied the germination of these seeds in the temperature range between 20⁰C and 35⁰C.

The main aim of the present study was to establish the responses in seed formation and seed production in several genotypes of tomatillo (*Physalis ixocarpa* Brot.) with a target to predict the seed yield and its realization, and at the same time to supplement the existing information about plant development throughout their life cycle.

MATERIALS AND METHODS

The experiments in this study were carried out in South Bulgaria conditions in 2016-2018 years at the Agricultural University-Plovdiv, Bulgaria, in his scientific field and also in scientific laboratories of the Department of Horticulture with the following genotypes and breeding lines of tomatillo (*Physalis ixocarpa* Brot.) with different origin:

018-2010 – origin from Colombia;

09-2012 – origin from Venezuela;

10-2012 – origin from Mexico;

14-2013 – origin from Mexico;

22-2015 – origin from China.

The plants were grown by unpricked seedlings. These seedlings were grown in a plastic unheated greenhouse and term of sowing was March 15th. The seed rate per one hectare was 80-100 g, but per square meter was sown at 1.5 g. The planting done in May 20-25, depends on the environmental conditions during different years. The scheme for planting was on furrows, on the scheme 70 x 50 cm, according to the previous investigation and recommendation (Panayotov & Tcorlianis, 2000). All necessary agro-technological practices were implemented in the optimum time and volume. The experiments carried out in four replicates with 25 plants, with an experimental plot from 9 m². At the stage of full botanical maturity, all the ripe fruits were harvested and the fruit yield was established. After that, the seeds were extracted throughout fermentation. The seed

yield was determined. The quantity of seed in one kilogram of fruits was established. The ratio between these fruits (one kilogram) and the seeds inside these fruits also was calculated. In other 5 fruits from each replicates all seeds were extracted and their number was counted.

In purpose to determine the seed development behaviours, the following indexes: the insemination by the percentage of fully developed seeds in one fruit to the quantity of a total number of seeds per fruit was registered. The linear seed sizes (length, width and thickness of seed) on 15 seeds with electronic calliper; the weight of one seed - set on 15 seeds, the weight of 1000 seeds (ISTA, 2013), in 4 replicates were determined. The sowing quality of the seeds were established by the following parameters: germinating energy (ISTA, 2013), in 4 replicates; germination (ISTA, 2013) in 4 replicates; uniformity of germination (according to Strona, 1966); mean germination time (MGT) - in four replicates, each of 100 seeds.

in addition, in order to gain a better understanding of the differences between the studied genotypes in the sowing qualities the fresh mass of a one seedling, by measuring of all developed seedlings; length of the embryo root and the hypocotyl - by measuring 10 seedlings from each replicates on the day of counting of germination in the four replicates were recorded. The deviations from the normal structure of the seedlings - short embryo root, lack of branches in embryo root, lack of hairs on the embryo root, undeveloped cotyledons, unopened cotyledons, lack of hypocotyls, by Welington (1970) were established. Data of the study were subjected to ANOVA test and correlation coefficients between some parameters also were established (Fowel & Cohen, 1992). The trend of the results of all vegetation are similar, therefore the presented data are average values from three years of study.

RESULTS AND DISCUSSIONS

Some characteristics of tomatillo seed formation are presented in Table 1. Genotypic differences were established about the number of seeds that setting up in one fruit, as the significant variation was established and it

ranging from 105.2 for genotype 09-2012 (Venezuela) to 154.7 for genotype 14-2013, originating from Mexico. Relatively smaller numbers of seed were developed in breeding line 22-2015 (112.6) numbers. The average numbers of seeds per fruit for all tested genotypes are 128.9. The variation to this among genotypes with the lowest and highest number of seeds is between -23.7 and +26.7. The difference between the highest and the lowest result, for seed number is even greater, almost double, 50.4 numbers. The differences between the variants are with statistical significance with exception of those between 10-2012 and 14-32013. Higher differences are observed in the weight of seeds in one fruit. Average in tomatillo fruit the content of seeds are approximately 83.4 mg. The highest weight of seed was established in genotype 14-2013 (102.7 mg), followed by 10-2012 with 89.0 mg, both originating from Mexico. The lowest seed weight per tomatillo seed established in № 09-2012, which is from Venezuela, only 59.0 mg.

The strong positive correlation was calculated between the number and weight of seeds per fruit, with correlation coefficient $r = +0.79$.

Not only the number of seeds is important, but the most significant for a better understanding of seed formation is also the percentage of fully developed seeds. In almost all tested tomatillo genotypes, the most seeds are fully developed. These which originate from Mexico and Venezuela - 10-2012, 14-2013 and 09-2012 reached up to 100.0%. In 018-2010 the percentage of the fully developed seed is weaker (87.1%) as in this genotype the number of set seeds, found in the fruit was also relatively lower. The pollination and fertilization have a strong influence on the setup and development of the seed. Peña-Lomeli et al. (2018) found that the highest efficiency of tomatillo seed formation and production is obtained in the condition of good pollination and they also observed high genotype response.

Table 1. Tomatillo seed formation

№	Genotype	Seeds number/fruit	Weight of seed/fruit (mg)	% of normal developed seeds	Weight of 1000 seeds (g)
1	018-2010	116.3	87.7	87.1	0.927
2	09-2012	105.2	59.0	100.0	0.867
3	10-2012	155.6	89.0	99.1	1.005
4	14-2013	154.7	102.7	100.0	1.013
5	22-2015	112.6	78.8	98.8	0.940
	average	128.9	83.4	97.0	0.950
	LSD $p = 0.05\%$	6.4			0.098
	$r = +0.79$				

r - correlation coefficient between number and weight of seeds per fruit.

The description of seed sowing quality is complemented by the weight of 1000 seeds. The variations in this behaviour are low. The highest weight of 1000 tomatillo seeds was measured in 14-2013 (1.013 g), followed by 10-2012 (1.005 g). The lowest, with difference the highest values from 0.146 g was the weight of 1000 seeds of line 09-2012 (0.867 g), even though the above mentioned the highest percentage of fully developed seeds - 100%. The low weight of 1000 seed was established also for 018-2010 (0.927 g). The average weight of 1000 tomatillo seeds is 0.950 g and variation between both the limit values established in the study is from -0.083 g to +0.063 g.

Statistical significances are established about the weight of one seed (Table 2). The average weight of one tomatillo seed is 0.71 mg. Between the investigated genotype and breeding lines it is within wide limits and changes from 0.35 mg in 22-2015 to 1.38 mg for 09-2012. Somewhat this tendency is also preserved in relation to the length of the seed. This sign varies between 1.49 mm to 2.22 mm in 14-2013 and in 10-2012, respectively, while the average value is 1.84 mm. More close are the results about seed width – from 1.24 mm to 1.81 mm in the above-mentioned genotypes and the deviation from the average wide is -0.26 mm and +0.31 mm. A similar tendency was also observed for the thickness of one seed,

that is from 0.16 mm (09-2012) to 0.44 mm (10-2012), which is with 48.0% less and with 57.14% more in comparison with the average value (0.28 mm). More of differences are statistically proved. About the importance of the seeds, morphology reported Zhang and Wen (1996) and point out that the seed behaviours can be used as an indicator for

taxonomic identification between species and genotypes of the genus *Physalis*. Between the investigated lines in these conducted experiments, some differences in seed size were established. Inzunza et al. (1999) also observed some genotype differences in the tomatillo seed dimensions.

Table 2. Morphology behaviours of one tomatillo seed

Nº	Genotype	Weight (mg)	Length (mm)	Width (mm)	Thickness (mm)
1	018-2010	0.76	2.21	1.70	0.38
2	09-2012	1.38	1.68	1.36	0.16
3	10-2012	0.62	2.22	1.81	0.44
4	14-2013	0.42	1.49	1.24	0.22
5	22-2015	0.35	1.62	1.40	0.26
	Average	0.71	1.84	1.50	0.28
LSD p = 0.05%		0.06	0.15	0.21	0.04

Germination is the most important for characterization of the seed as a sowing material. From carried out experiments each genotype is with high germination energy (Table 3). The highest energy of germination was observed in genotype 09-2012 (96.1%) followed by this of 10-2012 (90.0%). The lowest one was in line 22-2015 (65.5%). As Balck et al. (2008) and Copeland and McDonalds (2001) emphasized the seeds that have demonstrated higher germinating energy are with better vital performance because they have germinated for a shorter period.

Da Silva et al. (2016) reported that between each species in the genus *Physalis* the seeds from *Physalis ixocarpa* Brot. are with the lower percentage of emergence, while according to Thomson and Wit (1987) and Panayotov (2018) the seed from *Physalis peruviana* L. are characterized with high germination. Notwithstanding these statements in the carried out study good and high germination of tomatillo seeds was established. The highest one was also for Venezuelan genotype - 09-2012 (97.3%) which is with 12.1% more than average data. In the next place is the germination of Mexican genotype 10-2012 (92.0%). The lowest one was the germination of genotype 018-2010 (76.4%). The most intense, in the period between the two accounts, were the germination processes in 22-2015, where the difference between germinating energy and germination was 13.3%. In this genotype as mentioned above the germination

energy was the lowest. The results are with statistical significance.

The indicators mean germination time and uniformity of germination (Table 4) promote for more prices study of the seed qualities. The highest mean germination time was registered in genotype 10-2012 (2.24 days) and the lowest one, the most slowly germinated seeds, was in 22-2015 (8.40 days). Average for germination of one tomatillo seeds are necessary 4.76 days. Between other genotypes about this sign the response did not establish. More significant are the differences in uniformity of germination. Black et al. (2008) and Panayotov (2015) maintains, that this characteristic of seeds is important because it is related to the possibilities the seed to overcome easier the resistance of soil during sprouting. This characteristic range from 5.18% to 11.25% in 22-2015 and in 10-2012, respectively. Considering the average values of 7.81%, it can indicate that the tomatillo seeds are characterized by low uniformity of germination. The low germination at 22-2015, mentioned above, may be due to the significantly lower values of these two indices. In the study of sixteen tomatillo genotypes, Sánchez et al. (2007) found strong differences in parameters that related to the seed vitality.

The morphological features of the seedling (Table 5) provide additional information about the seed vitality status. Statistically significant differences were registered about the fresh weight of the seedling. In the fresh weight of the

seedling of one seed, the differences are more significant. Copeland and Mc Donalds (2001) and also Panayotov (2015) reported that the weight of the seedling very often applied in several methods for determining the vigour as well as it demonstrates the opportunity for easier germination of the seeds. The average weight of tomatillo seedling is 10.86 mg and for the investigated genotype it is in big diapason from 5.9 mg to 11.9 mg in 22-2015 (from China) and 10-2012 (from Mexico). The correlation coefficient between mean germination time and seedling weight is middle but negative with $r = -0.55$. It can be assumed that as the germination time is shorter, the period of seedling development, to the end of germination, is longer, which eventually contributes to the accumulation of higher biomass and vice versa at a lower mean germination time. Therefore this correlation coefficient is negative but strong. Sánchez et al.

(2007) and Martínez et al. (2006) also reported about the positive correlation with speed of germination and emergence, shoot and root length, and seedling dry weight. The highest length of the hypocotyls have developed the seeds from № 018-2010 (2.76 cm) and the lowest one was in 22-2015 (1.47 cm). The differences of these results to the average value are +0.69 cm and -0.6 cm. Long hypocotyls have developed also the seeds from 09-2012 and 10-2012. Genotype 22-2015 had developed also the lowest length of embryo root 2.81 cm, which are with 1.48 cm lower than the average values. The seedlings from Mexican genotype 14-2013 are with the highest embryo root (5.48 cm), followed by these of 10-2012, also from Mexico and 018-2010, which is from Colombia. These results unambiguously show that the variation between morphological behaviours of tomatillo seedlings for investigated genotypes is strong.

Table 3. Germination behaviours of tomatillo seeds

№	Genotype	Germination energy (%)	Germination (%)
1	018-2010	74.7	76.4
2	09-2012	96.1	97.3
3	10-2012	90.0	92.0
4	14-2013	80.7	81.3
5	22-2015	65.5	78.8
	average	81.6	85.2
LSD p = 0.05%		5.5	4.2

Table 4. Sowing parameters of tomatillo seeds

№	Genotype	MGT (days)	Uniformity (%)
1	018-2010	4.42	7.56
2	09-2012	4.04	6.95
3	10-2012	2.24	11.25
4	14-2013	4.70	8.12
5	22-2015	8.40	5.18
	average	4.76	7.81
LSD p = 0.05%		1.28	1.12

Table 5. Morphological characteristics of tomatillo seedling

№	Genotype	Fresh weight (mg)	Length of hypocotyl (cm)	Length of embryo root (cm)
1	018-2010	17.7	2.76	4.59
2	09-2012	9.2	2.39	3.77
3	10-2012	11.9	2.16	4.83
4	14-2013	9.6	1.59	5.48
5	22-2015	5.9	1.47	2.81
	average	10.86	2.07	4.29
LSD p = 0.05%		2.4	0.12	1.06
		$r = -0.551$		

r - correlation coefficient between fresh weight and MGT.

Not each seed developed normal seedlings. Some of them are characterized by different deviations from their normal morphology (Table 6). Average values of abnormally seedling grown between studied genotypes are 12.2%. This percentage of deviation was the highest in № 10-2012 followed by № 14-2013 with 13.5% and 12.8%, respectively. The

lowest one, it was recorded in genotype 22-2015 (11.1%). The most common deviations of seedling developed, calculated towards all, not well-developed seedlings which were accepted for 100%, are the lack of branches of the embryo with 26.4% and also undeveloped cotyledons with 22.1%. The highest percentage of seedlings with lack of branches on embryo

root was observed in genotype 09-2012 (36.2%), followed by these of 10-2012 and 14-2013 with 27% approximately. The deviation undeveloped cotyledons ranged from 0% in 09-2012 to 35.3% in 22-2015. The lowest

abnormal seedling are these with unopened cotyledons, average - 5.3% and it was presented only in genotype 09-2012 (26.4%), followed by these with lack of hairs on embryo root - 14.6%.

Table 6. Deviation from normal developed tomatillo seedlings (%)

Genotypes	%	Short embryo root	Lack of branches on embryo root	Lack of hairs on embryo root	Undeveloped cotyledons	Unopened cotyledons	Lack of hairs on hypocotyls
018-2010	12.2	32.8	18.3	22.2	26.7	0.0	0.0
09-2012	11.4	0.0	36.2	19.8	0.0	26.4	17.6
10-2012	13.5	10.2	27.4	18.2	26.9	0.0	17.3
14-2013	12.8	29.3	27.9	0.0	21.8	0.0	21.0
22-2015	11.1	13.8	22.2	11.6	35.3	0.0	17.1
Average	12.2	17.2	26.4	14.4	22.1	5.3	14.6

The main assessment of a given genotype or agrotechnological event is related to production, which determines its agronomic and economic significance. In order to predict the expected seed yield, the seed quantity in one kilogram of fruits in botanical maturity was determined, and it was an average of 7.39 g (Table 7) for the studied genotypes. The highest quantity of the seed in one kilogram of fruit was established for 10-2012 (8.10 g) and on the next place is 14-2013 with 7.72 g, both genotypes from Mexico. The values of this index are low about genotypes 22-2015 and 05-2012 (6.73 g and 6.85 g), respectively.

Through the ratio between the fruit and the extracted from them seeds are achieved in full assessment of the insemination. This also indicates what is the quantity of fruits from which can be obtained necessary seeds. The variation in this sign between investigated genotype is very high, the values are in diapason from 123.4 for 10-2012 to 148.6 for 22-2015, while the average ratio is 135.9.

The seed yield is in direct relation to the fruit yield. Therefore the quantity of harvested fruits is very important. The average fruits yield of the studied tomatillo population is 17411.0 kg.ha⁻¹. The highest one was obtained in the genotype 14-2013 (22422.8 kg.ha⁻¹), followed by 10-2013 (20265.2 kg.ha⁻¹). The lowest fruits yield was registered in 22-2015 and in 08-2010 (12012.2 kg.ha⁻¹ and 15651.2 kg.ha⁻¹), respectively. The differences are with mathematical significance. Statistical significance genotypic differences were

established about tomatillo seed yield in South Bulgarian conditions. The highest quantity of seeds is obtained from Mexican genotype 14-2013 (173.3 kg.ha⁻¹), which is more with 45 kg.ha⁻¹ to the average yield from each genotype. On second place is the № 10-2012 with 154.2 kg.ha⁻¹. This may be due to the high yields of fruit and low ratio fruit: seeds and also to the higher weight of 1000 seeds, to the greater number and weight of seeds per fruit as well as the high percentage of fully developed seeds in these two genotypes. This is confirmed also from the established strong and positive correlations between seed yield on one hand and their number of seeds per fruit ($r = +0.87$), a weight of the seeds in one fruit ($r = +0.65$) as well as the weight of 1000 seeds ($r = +0.70$) on the other hand. In № 22-2015 was registered the least seed yield from 80.9 kg.ha⁻¹, this genotype is characterized with the lowest fruit yield, the highest ratio fruits: seeds and also with the relatively low number of seed in one fruit and weight of 1000 seeds. The average seed yield of the tomatillo genotype in this experiments carried out under environmental conditions of South Bulgaria is 128.3 kg.ha⁻¹. The differences in seed productivity are statistically proven, except for that between genotypes 018-2010 and 09-2012.

The established results can be used both to predict the expected seed yield and to more accurately determine the commercial price, depending on genotypic characteristics. The price must be higher for samples with a lower seed yield and a higher fruit : seeds ratio, since

the input costs for fruit production, are almost the same for each genotype, but for those with low seed yields there are additional incurred

expenses about the extraction of higher quantities of fruits to be obtained the adequate amount of seed.

Table 7. Productivity behaviours of tomatillo genotypes

№	Genotype	Seed yield/ 1 kg fruits (g)	Ratio fruit: seeds	Yield of fruit (kg.ha ⁻¹)	Yield of seeds (kg.ha ⁻¹)
1	018-2010	7.57	132.1	15651.2	118.6
2	09-2012	6.85	145.9	16683.6	114.4
3	10-2012	8.10	123.4	20265.2	154.2
4	14-2013	7.72	129.5	22442.8	173.3
5	22-2015	6.73	148.6	12012.2	80.9
	Average	7.39	135.9	17411.0	128.3
LSD p = 0.05%		0.78		1046.3	15.98
r with number of seeds/fruit					r = +0.87
r with weight of seeds/fruit					r = +0.65
r with weight of 1000 seeds					r = +0.70

CONCLUSIONS

In indexes, the number of seeds and their weight in one fruit of tomatillo the genotypic differences were found. The percentage of fully developed seeds in each tested tomatillo genotypes is high. The differences of the tomatillo seed size have also been established. Each vitality indicator of tomatillo seeds is characterized by high variation between the genotypes.

The differences in the fresh weigh of one seedling are more significant. Most often the type of deviations from the normal development of seedling included the lack of branches on embryo root and undeveloped cotyledons. Genotypic differences are also noted in insemination. The seed weight in one-kilogram tomatillo fruits as well as fruit: seeds ratio changes in a wide range.

The variations between individual genotypes about fruit and seed yield are significant. The average tomatillo seed yield between tested genotypes under South Bulgarian environmental conditions is 128.3 kg.ha⁻¹. Under these conditions, Mexican genotypes, 14-2013 and 10-2012, are performing with the highest productivity.

The strong positive correlations between the number and weight of the seed per fruit, as well as about seed yield with the number and weight of the seeds in one fruit and the weight of 1000 seeds and middle negative correlation between the seedling fresh weight and MGT, were established.

The conducted studies supplement the information on the overall development of tomatillo plants, especially by the seed development, the scope where the knowledge is quite limited.

The presented results are suitable to be applied for prediction of the expected yield of tomatillo seeds as well as to precise the realization price, depending on the genotyping insemination characteristics. It can be assumed that this will help be increasing the efficiency of the production of tomatillo seed.

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